

CLAIMS

1. A method of analyzing the C-terminal amino acid sequence of the peptide, comprising:

obtaining C-terminal-deleted peptides lacking amino acid residues from said C-terminal by degrading the amino acids from said peptide C-terminal sequentially;

measuring the molecular weight of said C-terminal-deleted peptides; and

determining the decrease in molecular weight associated with said sequential degradation from the difference between the molecular weight obtained in said measuring the molecular weight of the C-terminal-deleted peptides and the molecular weight of said peptide, and analyzing said C-terminal amino acid sequence based on the decrease in said molecular weight,

wherein said C-terminal amino acids are degraded by making said peptide substantially bring into contact with an alkanoic anhydride in said obtaining C-terminal-deleted peptides.

2. The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 1, further comprising measuring the molecular weight of said peptide,

wherein said analyzing the amino acid sequence enables the decrease in molecular weight associated with the sequential degradation to be determined from the difference

between the molecular weight obtained in said measuring the molecular weight of the peptide and the molecular weight obtained in said measuring the molecular weight of
10 C-terminal-deleted peptides.

3. The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 1 or 2, further comprising allowing water molecules to act on said C-terminal-deleted peptides after said obtaining the
5 C-terminal-deleted peptides and before said measuring the molecular weight of the C-terminal-deleted peptides.

4. The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 3,
wherein said allowing water molecules to act includes bringing said C-terminal-deleted peptides into contact with
5 an aqueous solution containing a basic nitrogen-containing compound or a tertiary amine.

5. A method of analyzing the C-terminal amino acid sequence of the peptide, comprising:

obtaining C-terminal-deleted peptides lacking amino acid residues from said C-terminal by degrading the amino
5 acids from said C-terminal of said peptide sequentially;

obtaining C-terminal-deleted peptide-derived peptide fragments by cleaving said C-terminal-deleted peptides at predetermined positions;

measuring the molecular weight of said
10 C-terminal-deleted peptide-derived peptide fragments;
determining the decrease in molecular weight
associated with said sequential degradation from the
difference between the molecular weight obtained in said
measuring the molecular weight of C-terminal-deleted
15 peptide-derived peptide fragments and the molecular weight
of the peptide fragments obtainable from said peptide and
analyzing said C-terminal amino acid sequence based on said
decrease in molecular weight,
wherein said C-terminal amino acids are degraded by
20 making said peptide substantially bring into contact with
an alkanolic anhydride in said obtaining C-terminal-deleted
peptides.

6. The method of analyzing the C-terminal amino acid
sequence of the peptide according to Claim 5, further
comprising:

obtaining peptide-derived peptide fragments by
5 cleaving said peptide at said predetermined positions; and
measuring the molecular weight of said peptide-derived
peptide fragments,

wherein the decrease in molecular weight associated
with said sequential degradation is determined from the
10 difference between the molecular weight obtained in said
measuring the molecular weight of the peptide-derived
peptide fragments and the molecular weight obtained in said

measuring the molecular weight of C-terminal-deleted peptide-derived peptide fragments.

7. The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 5 or 6,

wherein said obtaining the C-terminal-deleted peptides includes protecting particular amino acid residues
5 in said peptide and thus eliminating the susceptibility of said particular amino acid residues to said cleavage in said obtaining the C-terminal-deleted peptide-derived peptide fragments.

8. The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 7,

wherein said obtaining the C-terminal-deleted peptide-derived peptide fragments includes treating said
5 C-terminal-deleted peptides with a protease.

9. The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 8,

wherein said protease is trypsin, and said eliminating the susceptibility of the particular amino acid residues
5 includes N-acylating said peptide.

10. The method of analyzing the C-terminal amino acid sequence of the peptide according to any one of Claims 7 to 9,

wherein said protection is O- and N-acylation of said
5 peptide and the protecting groups by O-acylation are
deprotected after said obtaining the C-terminal-deleted
peptide and before said obtaining the C-terminal-deleted
peptide-derived peptide fragments.

11. The method of analyzing the C-terminal amino acid
sequence of the peptide according to any of Claims 5 to 10,
wherein said measuring the molecular weight of
C-terminal-deleted peptide-derived peptide fragments
5 includes performing mass spectrometric measurement based
on cationic and anionic species; and

said analyzing the amino acid sequence from the
C-terminal includes identifying said C-terminal-deleted
peptide-derived peptide fragments associated with said
10 C-terminal of said peptide by comparing the mass
spectrometric results based on cationic species and the mass
spectrometric results based on anionic species.

12. The method of analyzing the C-terminal amino acid
sequence of the peptide according to any of Claims 5 to 11,
further comprising allowing water molecules to act on said
C-terminal-deleted peptides after said obtaining the
5 C-terminal-deleted peptides and before said obtaining the
C-terminal-deleted peptide-derived peptide fragments.

13. The method of analyzing the C-terminal amino acid

sequence of the peptide according to Claim 12,

wherein said allowing water molecules to act includes bringing said C-terminal-deleted peptides into contact with
5 an aqueous solution containing a basic nitrogen-containing aromatic ring compound or a tertiary amine.

14. The method of analyzing the C-terminal amino acid sequence of the peptide according to any one of Claims 5 to 13,

wherein said obtaining the C-terminal-deleted
5 peptides is carried out while said peptide is retained in the gel.

15. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 1 to 4,

wherein the processings prior to said measuring the molecular weight of the C-terminal-deleted peptides are
5 performed in the gel.

16. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 5 to 13,

wherein the processings prior to said measuring the molecular weight of C-terminal-deleted peptide-derived
5 peptide fragments are performed in the gel.

17. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 14 to

16, further comprising crosslinking said peptide before said obtaining the C-terminal-deleted peptides.

18. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 14 to 17, further comprising isolating said peptide from the peptide-containing mixture by polyacrylamide gel electrophoresis before said obtaining the C-terminal-deleted peptides,

wherein said obtaining the C-terminal-deleted peptides is performed while said isolated peptide is retained in said gel used in said polyacrylamide gel electrophoresis.

19. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 14 to 18,

wherein said obtaining the C-terminal-deleted peptides includes immersing said gel in a solution of an alkanoic anhydride in a dipolar aprotic solvent.

20. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 1 to 19,

wherein said obtaining the C-terminal-deleted peptides is carried out in a system containing a basic nitrogen-containing aromatic ring compound.

21. The method of analyzing the C-terminal amino acid

sequence of the peptide according to Claim 20,

wherein said basic nitrogen-containing aromatic ring compound is a pyridine base or the derivative of the pyridine
5 base.

22. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 1 to 21,

wherein said alkanoic anhydride is the symmetric anhydride of an alkanoic acid of 2 or more and 6 or less
5 carbon atoms.

23. The method of analyzing the C-terminal amino acid sequence of the peptide according to any of Claims 1 to 22,

wherein said alkanoic anhydride is the symmetric anhydride of a straight-chain alkanoic acid of 2 or more
5 and 6 or less carbon atoms.